## REMARKS/ARGUMENTS

Claims 1-13 and 15 remain in this application. Claim 14 has been cancelled by the present amendments. Claim 5 has been amended.

### *Concerning 35 USC § 112 Second Paragraph*

Claims 5, 6 and 14 stand rejected under 35 USC § 112, second paragraph, as being indefinite for the recitation of trade-mark items.

Claim 14 has been cancelled and claim 5 (and therefore dependent claim 6) have been amended to no longer recite a trade-mark item.

### *Concerning 35 USC § 103(a)*

Claims 1-15 of record stand rejected under 35 USC 103(a) as being unpatentable over Nash *et al. (1985) J. Reprod. Immunol.* 7:151-162 and Alving *et al.* U.S. 6,110,492, taken with Glenn *et al.* (U.S. 5,980,898)), Gupta *et al.* (1993) *Vaccine*, 11/13:293-306, and Edelman *et al.* (1990) *Intern. Rev. Immunol.* 7/1:51-66.

The Examiner contends that both Nash *et al.* and Alving *et al.* teach the instantly claimed invention with the exception of the feature that the antigen is encapsulated in the liposome. The Examiner contends that Glenn *et al.* teach a formulation comprising an antigen and an adjuvant and that a hydrating agent such as liposomes, water-in-oil emulsions or oil may be present, and also that the antigen can be encapsulated in the liposome. The Examiner further states that Gupta *et al.* and Edelman *et al.* teach antigens incorporated into liposomes.

The Examiner concludes that it would have been obvious to combine the components as taught in Nash *et al.* and Glenn *et al.*, or in Alving *et al.* and Glenn *et al.* to make a composition comprising a carrier (i.e. water-in-oil emulsion or oil), a liposome, an antigen encapsulated in the liposome and an adjuvant. The Examiner states that Glenn *et al.*, Gupta *et al.* as well as Edelman *et al.* all teach the use of various antigens as well as the encapsulation of those antigens in the liposome. The Examiner concludes that Gupta *et al.* teach that the immune response was better if the antigen were encapsulated in the liposome and that therefore it would have been obvious to encapsulate the antigen in the liposome to increase the immune response or vaccine protection of the composition.

Applicants traverse this rejection and submit that the claims patentably distinguish from the cited references or any combination thereof. The references do not teach or suggest, either individually or in combination, Applicants' claimed invention, which is a vaccine composition, comprising:

(a) a carrier comprising a continuous phase of a hydrophobic substance;

(b) liposomes;

(c) an antigen encapsulated in said liposomes, said antigen being an antigen which, when not in said vaccine composition, has a conformation other than its native conformation, with the proviso that said antigen is other than a zona pellucida-derived antigen; and,

(d) an adjuvant.

Nash *et al.*

The Examiner contends that Nash *et al.* teach a composition comprising an antigen, a water-in-oil carrier, an adjuvant, and liposomes and that the only feature of claim 1 not found in Nash *et al.* is the antigen being encapsulated in the liposome.

Applicants disagree. Not only does Nash *et al.* not teach antigens encapsulated or incorporated into liposomes, importantly, Nash *et al.* do not teach or suggest the combination of a water-in-oil carrier (i.e. a carrier comprising a continuous phase of a hydrophobic substance) and liposomes.

Nash *et al.* tested in rabbits a variety of formulations of potential anti-pregnancy vaccines based on the β-subunit of human chorionic gonadotropin. The various formulations tested are described in the text of Nash *et al.* at the paragraph bridging pages 157 and 158 and the results and descriptions of the formulations are shown in Table 4 on page 158. As discussed in the paragraph bridging pages 157 and 158, Nash *et al.* tested water-in-oil emulsions, oil-in-water emulsions, commercial oil-in-water emulsions, liposomes, and formulations containing lecithin and glycerol.

Importantly, each of these formulations are described as being separate and distinct. There is no indication that the liposomes were put in a water-in-oil carrier. In Table 4, which indicates whether the continuous phase is oil (i.e. hydrophobic) or aqueous, it is shown that in the liposome formulation no emulsifier was used, and a continuous phase is shown as being absent by the presence of a hyphen. That is, not only does Table 4 indicate that the continuous phase in the liposome formulation is neither oil nor an aqueous continuous phase, but the presence of the hyphen shows that the liposome formulation would not be construed as having a continuous phase. In fact, the title of Table 4 uses the term "aqueous suspension" suggesting that liposomes were suspended in water with no oil phase at all.

The liposome formulation is discussed in Nash *et al.* completely separately from all other formulations, and there is no discussion whatsoever in Nash *et al.* that liposomes could be incorporated into one of the other formulations, let alone a water-in-oil formulation.

As discussed at pages 158-159, Nash *et al.* found that the liposome formulation gave only an intermediate response. Moreover, Figures 1 and 2 of Nash *et al.* show that antibody titers had diminished by 24 weeks post-immunization such that they were no greater than titers obtained

using an aqueous suspension. In contrast, the instantly claimed invention produces titers which are of significantly longer duration. For example, titers are undiminished in grey seals and white-tail deer for ten and four years respectively. As discussed in the enclosed Declaration under 37 CFR §1.132 of inventor Robert Brown, Ph.D., undiminished titers have been demonstrated for up to 20 months in rabbits, i.e. the same animal used in the Nash *et al.* study.

One can therefore conclude that Nash *et al.* used a formulation wherein the liposomes were in an aqueous carrier, not a carrier comprising a continuous phase of a hydrophobic substance as instantly claimed. Were it otherwise, Nash *et al.* would presumably have obtained better results, as in the instant invention.

Moreover, prior to Applicants' invention, the use of liposomes in combination with a carrier comprising a continuous phase of a hydrophobic substance was counter-intuitive. The skilled person would expect that the liposomes would be soluble in the oily carrier and consequently degrade in the oily carrier, rendering the liposomes useless. The liposomes would be expected to retain their integrity only in an aqueous carrier, in which the liposomes are insoluble. Applicants were surprised to find that they were able to formulate vaccine compositions comprising liposomes in a carrier comprising a continuous phase of a hydrophobic substance, in which the liposomes did not dissolve or deteriorate. It is thought that Applicants' use of liposome formulations comprising a high ratio of phospholipid to sterol results in liposomes in which the outer surface of the bi-layer membrane is sufficiently hydrophilic to attract and retain a thin aqueous outer layer that prevents the lipid bi-layer of the liposome from contacting the hydrophobic carrier, thus preventing the liposomes from being dissolved in the hydrophobic carrier.

As discussed in the instant specification at, e.g. page 10 lines 23-30, page 16 lines 16-24 and page 19 lines 17-25, it is preferred that the sterol (e.g. cholesterol), is present in an amount of about 10% of that of the phospholipid, e.g. the cholesterol is present in an amount of about 0.01 g/dose of vaccine composition and the phospholipid is present in an amount of about 0.1 g/dose of the vaccine composition. As compared to the liposomes used by Nash *et al.*, Applicants' preferred composition is heavily weighted towards the phospholipid component and away from the cholesterol component. In contrast, as described by Nash *et al.* at page 153 lines 7 and 8: "Liposomes were prepared using *equamolar* quantities of DL-α-phosphatidylcholine, dipalmitol, and cholesterol." (emphasis added)

Therefore, even had Nash *et al.* used liposomes in a water-oil-formulation (of which there is no evidence), this presumably would not have worked as the liposomes as formulated by Nash *et al.* would have dissolved into the oily carrier.

Alving *et al.*

Alving *et al.* were concerned with stabilizing *oil-in-water* emulsions. Alving *et al.*'s solution was to use smectic mesophase vesicles (i.e. liposomes) and disintegrated forms thereof, as a stabilizing agent (see Alving *et al.* at e.g. column 2 lines 26-40).

Alving *et al.* indicate that the ratio of oil to water in the emulsion (i.e. carrier) is critical (see column 3 lines 35-41), and indicate that most preferably, the ratio of oil to water is about 10% oil/water (v/v) (see Alving *et al.* at column 3 lines 50-54). Thus, Alving *et al.* teach that the carrier must be an *oil-in-water* formulation, which is directly contrary to instant claim 1, which requires a continuous phase of a hydrophobic substance. Thus, Alving *et al.* teach away from the instantly claimed use of a carrier comprising a continuous phase of a hydrophobic substance.

Moreover, Alving *et al.* hypothesize that their oil-in-water emulsions were stabilized by the liposomes because a portion of the liposomes disintegrate, releasing amphiphilic components as endogenous emulsifiers (see Alving *et al.* column 4 lines 64-67). Thus, not only do Alving *et al.* require an oil-in-water carrier, the liposomes are present only to stabilize the emulsion by partially disintegrating. Alving *et al.* contemplate only compositions in which oil-in-water emulsions are stabilized with partially-disintegrating liposomes and Alving *et al.* do not teach or in any way suggest the combination of liposomes with a *water-in-oil* carrier.

Glenn *et al.*

Glenn *et al.* were concerned with accomplishing immunization through intact skin without the need for perforating the skin (see column 5 lines 46-51). The particular invention described and claimed in Glenn *et al.* is a patch for transcutaneous immunization comprising a dressing that causes hydration of intact skin, an immunizing agent and adjuvant (see e.g. claim 1 of Glenn *et al.*).

Glenn *et al.* do not appear to place any particular importance on the carrier at all let alone whether it is an oil-in-water or a water-in-oil carrier. As noted by the Examiner, Glenn *et al.* do state at column 3 lines 55-64 that, in addition to antigen and adjuvant, the formulation may comprise a hydrating agent such as liposomes, a penetration enhancer or both. In this passage concerning hydrating agents and penetration enhancers, Glenn *et al.* also mention emulsions (e.g. aqueous creams), oil-in-water emulsions (e.g. oily creams), anhydrous lipids and oil-in-water emulsions, anhydrous lipids and water-in-oil emulsions, fats, waxes, oil, silicones and humectants (e.g. glycerol). Thus, all of these materials are solely mentioned as various additives for hydrating the skin, presumably to aid penetration of the antigen and adjuvant. Glenn *et al.* do not distinguish between liposomes, oil-in-water emulsions or water-in-oil emulsions for this purpose.

At columns 11 and 12, Glenn *et al.* provide further details about the use of liposomes. At column 11 lines 57-60, Glenn *et al.* state that:

> Transcutaneous immunization may be achieved using liposomes. However, as shown in the examples, the liposomes are not required to elicit an antigen-specific immune response.

In column 12, Glenn *et al.* explain that antigens may or may not be encapsulated in the liposomes but state that it is preferred that the antigen is *not* encapsulated by the liposomes, contrary to the instant claims. Specifically, Glenn *et al.* state at column 12 lines 35-41:

> Liposomes may be formed as described above but without addition of antigen to the aqueous solution. Antigen may then be added to the pre-formed liposomes and, therefore, antigen would be in solution and/or associated with, but *not* encapsulated by, the liposomes. This process of making a liposome-containing formulation is *preferred* because of its simplicity. (emphasis added)

Thus, Glenn *et al.* actually teach away from the instant claimed invention as Glenn *et al.* make very clear that it is preferred that the liposomes be prepared with the antigen outside the liposome. In all of Glenn *et al.*'s working examples at columns 18-29, pre-formed liposomes were mixed with the antigen (see e.g. column 19 lines 5-13). Hence, in all cases, the antigen was outside the liposomes, contrary to the instant claims.

Moreover, Glenn *et al.* are entirely silent on the combination of a water-in-oil carrier and liposomes and do not suggest that this combination would be in any way advantageous. In each of Glenn *et al.*'s working examples, an *aqueous* carrier (saline) was used, and the carrier did not comprise a continuous phase of a hydrophobic substance as instantly claimed.

The use of an *aqueous* carrier in Glenn *et al.* was probably essential, in view of the technique of liposome formation described by Glenn *et al.* Unlike in the instant specification, where the liposomes were prepared using a high ratio of phospholipid to sterol (10:1) Glenn *et al.* describe molar ratio of 0.9:0.1:0.75 dimyristoyl phosphatidyl choline, dimyristoyl phosphatidyl glycerol, and cholesterol — i.e. a much higher proportion of the sterol. As discussed above, even had Glenn *et al.* taught or suggested a carrier comprising a continuous phase of a hydrophobic substance, the liposomes used by Glenn *et al.* probably would have dissolved, rendering the liposomes useless.

Gupta *et al.* and Edelman *et al.*

Gupta *et al.* and Edelman *et al.* do not cure the deficiencies in Nash *et al.*, Alving *et al.* and Glenn *et al.* Gupta *et al.* and Edelman *et al.* are review articles that add nothing new to the vaccine art. Both references confirm what is already known, that liposomes can be used in vaccines, and the antigen can be encompassed in the liposome, and that water-in-oil carriers can be used. But neither of these articles teach or suggest the *combination* of liposomes and a carrier comprising continuous phase of a hydrophobic substance, as instantly claimed. As discussed above, in the absence of any perceived advantage of combining these two elements, and with the knowledge that the oily carrier would presumably dissolve the liposomes, one would not be motivated to make this combination.

Accordingly, Applicants submit that the Examiner has not established the requirements for a *prima facie* case of obviousness. There is no motivation to combine or modify the Nash *et al.*, Alving *et al.* or Glenn *et al.* references, each of which in fact teach away from the instantly claimed invention for the reasons discussed above. Moreover, there is no reasonable expectation of success in making the presently claimed combination, as all of these references

describe liposome formulations that do not have a high ratio of phospholipids to sterol and the skilled person would therefore expect the liposomes to dissolve in the oily carrier.

Furthermore, the instantly claimed invention provides unexpected results. As discussed in the enclosed §1.132 Declaration of Dr. Robert Brown, the *combination* of liposomes and the water-in-oil carrier gave results that were surprisingly better than either (1) liposomes in an aqueous carrier or (2) a water-in-oil or oil carrier without liposomes.

As shown in Table B in Dr. Brown's Declaration, the combination of liposomes and an aqueous carrier gave much *lower* antibody titers than did a commercial hepatitis B vaccine (aqueous carrier/no liposomes). In contrast, the combination of liposomes and a carrier with a continuous hydrophobic phase gave much *higher* antibody titers than did the commercial vaccine. Given that liposomes alone actually provide a negative result, it is indeed surprising that when the liposomes are combined with the oil or water-in-oil carrier, very high antibody titers are obtained. These unexpected results could not have been predicted from the teachings of the cited references.

In view of the foregoing, Applicants respectfully request that the rejections be withdrawn and that a timely Notice of Allowance be issued in this case.

Respectfully submitted,

SMART & BIGGAR

By _____

David E. Schwartz
Reg. No. 48,211
Tel.: (613) 232-2486

Date: May 5, 2004

SMART & BIGGAR
P.O. Box 2999, Station D
900-55 Metcalfe Street
Ottawa, Ontario, Canada
K1P 5Y6

DES:srq:jed

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| Appl. No. | : 09/992,149 | Confirmation No. | 5280 |
| Applicant | : Robert Brown, et al | | |
| Filed | : 11/06/2001 | | |
| TC/A.U. | : 1645 | | |
| Examiner | : Minnifield, Nita M | | |
| Docket No. | : 84077 | | |
| Customer No. | : 07380 | | |

**DECLARATION PURSUANT TO 37 CFR § 1.132**

I, Robert Brown Ph.D. hereby declare that:

1. I am an inventor in the above-identified application.

2. Table 11 at page 47 of the patent application shows the production of anti-SIZP antibodies by rabbits immunized with vaccine formulations of the invention (Groups 1-4) and with control vaccines (Groups 5 and 6).

3. In *all* cases, the vaccines were formulated in a water-in-oil carrier, i.e. a carrier having a continuous hydrophobic phase. In some instances (groups 1, 2 and 6), the antigen was located in the saline droplets suspended in the continuous oil phase, and in some cases (groups 3, 4 and 5), the antigen was located in the continuous oil phase itself.

4. In groups 1-4, the antigen was contained in liposomes. In control groups 5 and 6, no liposomes were used.

5. The data in Table 11 show that the average anti-SIZP antibody titer one to three months postimmunization was much greater for the compositions containing liposomes (groups 1-4) than for the control groups without liposomes (groups 5-6).

6. Subsequently, we monitored the rabbits in groups 2 (liposomes) and 6 (no liposomes) (rabbit I.D. numbers 73, 42, 62, 77, 74 and 68,75 46 43) for antibody production for 20 months post-immunization (see Table A below). No statistical difference ($P = 0.21$) in antibody titers occurred in rabbits immunized with vaccines of the invention between 6 and 20 months post-immunization. In contrast, anti-SIZP antibody titers in the control group immunized without liposomes had fallen almost to zero by 20 months. Hence, it is apparent that, in contrast to conventional vaccines, vaccines of the invention have a long-lived effect, maintaining high antibody titers for at least 20 months.

## TABLE A

Production of anti-SIZP antibodies by rabbits immunized with the invention, SIZP in liposomes, alum outside liposomes and liposomes suspended in the aqueous phase of the saline-in-oil emulsion (50:50 oil/saline) or SIZP/alum placed in the aqueous phase of a 50:50 saline-in-oil emulsion (no liposomes).

| | Anti-SIZP titer (% of reference serum) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rabbit | Post-immunization (months) | | | | | | | | | | | | | |
| ID 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 13 | 16 | 20 |
| Invention (SIZP in liposomes, alum outside liposomes, in aqueous phase) | | | | | | | | | | | | | | |
| 73 0 | 373 | 273 | 304 | 241 | 306 | 167 | 138 | 176 | 136 | 125 | 198 | 163 | 131 | 136 |
| 42 0 | 328 | 199 | 281 | 218 | 194 | 125 | 107 | 156 | 105 | 116 | 167 | 187 | 116 | 130 |
| 62 0 | 259 | 194 | 244 | 359 | 334 | 162 | 153 | 150 | 116 | 121 | 189 | 141 | 131 | 128 |
| 77 0 | 182 | 131 | 235 | 230 | 280 | 169 | 107 | 165 | 130 | 172 | 210 | 149 | 150 | 136 |
| 74 0 | 295 | 238 | 225 | 290 | 184 | 105 | 95 | 114 | 90 | 74 | 133 | 98 | 100 | 89 |
| Conventional (SIZP/alum in aqueous phase – no liposomes). | | | | | | | | | | | | | | |
| 68 0 | 28 | 31 | 22 | 22 | 20 | 21 | 9 | 16 | 20 | 10 | 8 | 7 | 14 | 4 |
| 75 0 | 9 | 16 | 16 | 15 | 15 | 16 | 6 | 11 | 11 | 7 | 5 | 6 | 8 | 3 |
| 46 0 | 18 | 23 | 17 | 22 | 16 | 16 | 5 | 13 | 10 | 6 | 4 | 11 | 8 | 2 |
| 43 0 | 10 | 19 | 16 | 15 | 13 | 13 | 6 | 11 | 10 | 6 | 4 | 12 | 8 | 4 |

7. In another experiment, production of anti-Hepatitis B (anti-HepB) antibodies following inoculation of rabbits using a commercial vaccine (antigen/alum adjuvant/aqueous carrier) was compared to production of anti-HepB antibodies following inoculation using a vaccine formulated according to the invention (antigen and alum adjuvant encapsulated in liposomes/water-in-oil carrier). This experiment is described in detail in Example 10 in the patent application, wherein Table 10 at page 45 shows two of the treatment groups.

8. In addition, to these two groups, there was concurrently a third group of rabbits which were immunized with a vaccine in which the antigen was encapsulated in liposomes, but without a hydrophobic (i.e. oil) phase. Production of anti-HepB antibodies was substantially less in the absence of a hydrophobic phase despite the presence of liposomes (average titers were 336 vs. 2654 mlU/ml; mIU = milli International Units).

9. When the complete invention was employed, that is both liposomes and a hydrophobic phase were present, production of anti-HepB antibodies was enhanced. In fact, there was synergistic effect since liposomes by themselves (i.e. in an aqueous carrier) reduced antibody production relative to the commercial vaccine. The combination of liposomes and a hydrophobic phase greatly increased antibody production (about 6 times more antibody production than the commercial vaccine, average titers were 15,432 vs. 2,654 mlU/ml) indicating synergism in the use of liposomes with a hydrophobic phase. These results are set forth in Table B below.

## TABLE B

Production of anti-Hepatitis B (HepB) antibodies by rabbits immunized with HepB commercial vaccine, HepB vaccine formulated with all ingredients of the invention except oil or with a HepB vaccine formulated in accordance with the present invention.

| Rabbit ID | Anti-HepB titer (mlU/ml) Post-immunization (months) 0 | 1 |
|---|---|---|
| Commercial vaccine (no liposomes/aqueous carrier) | | |
| 96 | 0 | 736 |
| 101 | 0 | 1,237 |
| 97 | 0 | 488 |
| 100 | 0 | 1,877 |
| 99 | 0 | 6,251 |
| 103 | 0 | 8,384 |
| 98 | 0 | 688 |
| 102 | 0 | 1,568 |
| Average | 0 | 2,654 -/+ 1,050 |
| Vaccine of the invention but without oil (liposomes/aqueous carrier) | | |
| 104 | 0 | 48 |
| 91 | 0 | 144 |
| 94 | 0 | 48 |
| 87 | 0 | 48 |
| 89 | 0 | 1,968 |
| 90 | 0 | 48 |
| 92 | 0 | 48 |
| Average | 0 | 336 -/+ 272 |
| Vaccine of the invention (liposomes/carrier with continuous hydrophobic phase) | | |
| 93 | 0 | 32,341 |
| 95 | 0 | 3,371 |
| 88 | 0 | 5,717 |
| 81 | 0 | 23,808 |
| 83 | 0 | 9,344 |
| 84 | 0 | 17,856 |
| 79 | 0 | 9,344 |
| 85 | 0 | 21,675 |
| Average | 0 | 15,432 -/+ 3,573 |

10. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Robert Brown

Robert Brown Ph.D.

April 27, 2004

Date